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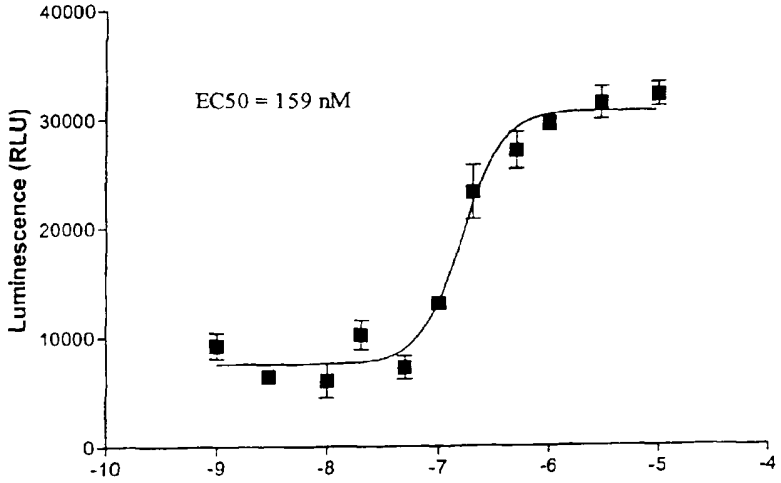
(54) Title: A RECOMBINANT CELL LINE EXPRESSING GPCRx11 AS A FUNCTIONAL RECEPTOR VALIDATED BY ANGIOPEPTIN AND USEFUL FOR SCREENING OF AGONISTS AND ANTAGONISTS



Dose response curve with angiopeptin

reference agonist. GPCRx11 cells gave a specific signal with synthetic angiopeptin and a somatostatin analog allowing to validate this cell line for screening of natural or synthetic agonists and antagonists. In parallel, extended tissue distribution and polyclonal antibodies have been produced to facilitate GPCRx11 characterisation.

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A RECOMBINANT CELL LINE EXPRESSING GPCRx11 AS A FUNCTIONAL RECEPTOR VALIDATED BY ANGIOPEPTIN AND USEFUL FOR SCREENING OF AGONISTS AND ANTAGONISTS

Field of the invention

[0001] The present invention is related to a newly identified member of the superfamily of G-protein-coupled receptors as well as to the various uses that can be made of said receptor.

[0002] The invention is also related to the polynucleic acid sequence (polynucleotide) encoding said receptor.

[0003] The invention is further related to methods using receptor polypeptide and polynucleotide applicable to diagnostic and treatment in receptor-mediated disorders.

[0004] The invention is further related to drug-screening methods using the receptor polypeptide and polynucleotide, to identify agonists and antagonists applicable to diagnostic, prevention and/or treatment of said various disorders.

[0005] The invention further encompasses unknown agonists and antagonists detected and recovered based on the receptor polypeptide and polynucleotide.

[0006] The invention is further related to procedures for producing the receptor polypeptide and

Background of the invention

[0007] G-protein coupled receptors (GPCRs) are proteins responsible for transducing a signal within a cell. GPCRs have usually seven transmembrane domains. Upon binding of a ligand to an extra-cellular portion or fragment of a GPCR, a signal is transduced within the cell that results in a change in a biological or physiological property or behaviour of the cell. GPCRs, along with G-proteins and effectors (intracellular enzymes and channels modulated by G-proteins), are the components of a modular signalling system that connects the state of intra-cellular second messengers to extra-cellular inputs.

[0008] GPCR genes and gene products are potential causative agents of disease and these receptors seem to be of critical importance to both the central nervous system and peripheral physiological processes.

[0009] The GPCR protein superfamily is represented in five families : Family I, receptors typified by rhodopsin and the beta2-adrenergic receptor and currently represented by over 200 unique members; Family II, the parathyroid hormone/calcitonin/secretin receptor family; Family III, the metabotropic glutamate receptor family, Family IV, the CAMP receptor family, important in the chemotaxis and development of *D. discoideum;* and Family V, the fungal mating pheromone receptor such as STE2.

[0010] G proteins represent a family of heterotrimeric proteins composed of α, β and γ subunits, that bind guanine nucleotides. These proteins are usually linked to cell surface receptors (receptors containing seven transmembrane domains).

[0011] Following ligand binding to the GPCR a

which caused the α-subunit to exchange a bound GDP molecule for a GTP molecule and to dissociate from the βγ-subunits.

[0012] The GTP-bound form of the α, β and γ-subunits typically functions as an effector-modulating moiety, leading to the production of second messengers, such as cAMP (e.g. by activation of adenyl cyclase), diacylglycerol or inositol phosphates.

[0013] Greater than 20 different types of α-subunits are known in humans. These subunits associate with a small pool of β and γ subunits. Examples of mammalian G proteins include Gi, Go, Gq, Gs and Gt. G proteins are described extensively in Lodish et al., *Molecular Cell Biology*, (Scientific American Books Inc., New York, N.Y., 1995), the contents of which are incorporated herein by reference.

[0014] Known and unknown GPCRs constitute now major targets for drug action and development.

[0015] Therefore, it exists a need for providing new G protein coupled receptors which could be used for the screening of new agonists and antagonists having advantageous potential prophylactic and therapeutical properties.

[0016] More than 300 GPCRs have been cloned thus far and it is generally assumed that it exists well over 1000 such receptors. Mechanistically, approximately 50-60% of all clinically relevant drugs act by modulating the functions of various GPCRs (Cudermann et al., *J. Mol. Med.*, Vol. 73, pages 51-63, 1995).

Summary of the invention

[0017] The present invention is related to newly identified member of G-protein-coupled receptor, preferably a human receptor, as well as to the polynucleotide sequence encoding said human receptor described hereafter (SEQ ID NO. 1 and 2).

[0018] The present invention is also related to other newly identified members of G-protein-coupled receptors, preferably human receptors, as well as to the polynucleotide sequence encoding said other human receptor described hereafter (SEQ ID NO. 3 to SEQ ID NO. 22).

[0019] The present invention is also related to nucleotidic and/or amino acid sequence homologous to the sequences corresponding to the receptor described hereafter.

[0020] An homologous sequence (which may exist in other mammal species) means a sequence which presents a high sequence identity or homology (which presents an identity higher than 70%, 75%, 80%, 85%, 90% or 95%) with the complete human sequence described hereafter, and preferably characterised by a similar pharmacology, especially a preference for binding angiopeptin and/or somatostatin analogs.

[0021] Another aspect of the present invention is related to a specific active portion of said sequence. Said active portion could be a receptor which comprises a partial deletion upon the complete nucleotide or amino acid sequence and which still maintains the active site(s) necessary for the binding of specific ligands able to interact with said receptor.

[0022] Homologous sequences of the sequence

specific human populations, but which are involved in the same biochemical pathway.

[0023] Such homologous sequences may comprise addition, deletion or substitution of one or more amino acids or nucleotides, which does not substantially alter the functional characteristics of the receptor according to the invention.

[0024] Thus, the invention encompasses also a receptor and corresponding nucleotide sequence having exactly the same amino acid or nucleotide sequences as shown in the enclosed sequence listing, as well as molecules which differ, but which are retaining the basic qualitative binding properties of the complete receptor according to the invention.

[0025] The invention is preferably related to said (human) receptor characterised by the complete nucleotide and amino acid sequences described hereafter, to unknown (and not previously described in the state of the art) agonist, reverse agonist and antagonist compounds or inhibitors of said receptor. Preferably, said inhibitors are antisens RNAs, rybozymes or antibodies (or specific hypervariable (FAB, FAB'2, ...) portions thereof) that bind specifically to said receptor or its encoding nucleotide sequence (i.e. that have at least a 10 fold greater affinity for said receptors than any other naturally occurring antibody). Said specific antibodies are preferably obtained by a process involving the injection of a pharmaceutically acceptable preparation of such amino acid sequence into a animal capable of producing antibodies directed against said receptor.

[0026] For instance, a monoclonal antibody directed

encoding said receptor into a mouse and than fusing mouse spleen cells with myeloma cells.

[0027] The present invention is also related to the polynucleotide according to the invention, possibly linked to other expression sequences and incorporated into a vector (plasmids, viruses, liposomes, cationic vesicles,...) and host cells transformed by such vector.

[0028] The present invention is also related to the recombinant, preferably human receptor according to the invention, produced by such host cells according to the method well known by the person skilled in the art, as well as a functional assay (diagnostic kit) comprising all the means and media for the identification of the receptor, its nucleotide sequence, as well as agonist, reverse agonist, antagonist and inhibitor of said receptor or its nucleotide sequence. Said diagnostic kit comprises preferably the following elements : the receptor, its encoding nucleotide sequence, antibodies directed against said receptor or its nucleotide sequence, as well as possible agonist, reverse agonist, antagonist or inhibitor compounds of said receptor. Said diagnostic kit comprises means and media for performing said diagnostic preferably through a measure of dosage/activity of said receptor, by genetic analysis of the receptor nucleotide sequence, preferably by RT/PCR or by immuno-analysis, preferably by the use of antibodies directed against said receptor.

[0029] The present invention is also related to a transgenic non-human mammal comprising a partial or total deletion of the genetic sequence encoding the receptor according to the invention, preferably a non human mammal comprising an homologous recombination "knock-out" of the

[illegible] above natural level said polynucleotide sequence.

[0030] Said transgenic non-human mammal can be obtained by methods well known by the person skilled in the art, for instance by the one described in the document WO98/20112 using classical techniques based upon the transfection of embryonic stem cells, preferably according to the method described by Carmeliet et al., *Nature*, Vol. 380, p. 435-439, 1996.

[0031] Preferably, in said transgenic non human mammal overexpressing, the polynucleotide according to the invention or active portions thereof has been previously incorporated in a DNA construct with an inducible promoter allowing its overexpression and possibly with tissues and other specific regulatory elements.

[0032] Another aspect of the present invention is related to a method and kit for performing said method for the screening (detection and possibly recovering) of compounds or a natural extract which are unknown (not yet described in the state of the art) or not known to be agonists, reverse agonists, antagonists or inhibitors of natural compounds to the receptor according to the invention, said method comprising :

- contacting a cell or cell extract from the cell transfected with a vector expressing the polynucleotide encoding the receptor according to the invention or active portion(s) thereof,
- possibly isolating a membrane fraction from the cell extract or the complete cell with a compound or molecules present in said natural extract under conditions permitting binding of said compound or said mixture of molecules to said receptor, possibly by the

said compound or said mixture of molecules to said receptor by means of a bioassay, (preferably a

modification in the production of a second messenger or an increase in the receptor activity) in the presence of another compound working as an agonist, reverse agonist, antagonist or inhibitor to the receptor according to the invention and thereby possibly recovering and determining whether said compound or mixture of molecules is (are) able to work as agonist, reverse agonist, antagonist, or inhibitor of the compound to its receptor.

[0033] Preferably, the second messenger assay comprises the measurement of intra-cellular cAMP, intracellular inositol phosphates, intra-cellular diacylglycerol concentrations, arachinoid acid concentration, MAP kinase(s) or tyrosine kinase(s) pathways activation or intra-cellular calcium mobilisation.

[0034] Preferably, said bioassay is validated by the addition of angiopeptin and any other suitable related peptides to the receptor according to the invention by a method well-known by the person skilled in the art and described hereafter.

[0035] The screening method according to the invention could be performed by well known methods to the person skilled in the art, preferably by high-throughput screening, diagnostic and dosage devices based upon the method described in the International patent application WO00/02045 performed upon various solid supports such as micro-titer plates or biochips (microarrays) according to known techniques by the person skilled in the art.

[0036] The present invention is also related to the known or unknown compound or molecules characterised and possibly recovered by said method for its (their) use as a

or molecule(s) and a pharmaceutically acceptable carrier or

diluent for the preparation of a medicament in the prevention and/or the treatment of various diseases.

[0037] In the pharmaceutical composition, the carrier or the adequate pharmaceutical carrier or diluant can be any solid, liquid or gaseous support which is non-toxic and adapted for the administration (in vivo or ex vivo) to the patient, including the human, through various administration roots such as oral administration, intravenous administration, intradermal administration, etc.

[0038] Said pharmaceutical composition may comprise also various vesicles or adjuvants well known by the person skilled in the art, able to modulate the immune response of the patient. The percentage of active compound-molecules/ pharmaceutical carriers can vary, the range being only limited by the tolerance and the efficiency of the active compounds to the patient. Said ranges of administration are also limited by the frequency of administration and the possible side effects of the compound or molecules.

[0039] A further aspect of the present invention is related to said unknown compound or molecule(s) identified by said screening method, to the pharmaceutical composition comprising it and to their use in the treatment of viral infections or diseases induced by various viruses or bacteria, the treatment or prevention of disturbances of cell migration, diseases or perturbations of the immune system, including cancer, development of tumours and tumour metastasis, inflammatory and neo-plastic processes, bacterial and fungal infections, for wound and bone healing and dysfunction of regulatory growth functions, pains, diabetes, obesity, anorexia, bulimia, acute heart failure, atherosclerosis, diseases characterised by excessive smooth

muscle cell proliferation, aneurysms, wound healing, diseases characterised by loss of smooth muscle cells or reduced smooth muscle cell proliferation, stroke, ischemia, ulcers, allergies, benign prostatic hypertrophy, migraine, vomiting, psychotic and neurological disorders, including anxiety, schizophrenia, maniac depression, depression, delirium, dementia and severe mental retardation, degenerative diseases, neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, and dyskinasias, such as Huntington's disease or Gilles de la Tourett's syndrome and other related diseases.

[0040] Among the mentioned diseases the preferred applications are related to therapeutic agents targeting 7TM receptor that can play a function in preventing, improving or correcting dysfunctions or diseases, including, but not limited to fertility, fœtal development, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV1 and HIV2, pain, cancer, anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, psychotic and neurological disorders including anxiety, depression, migraine, vomiting, stroke, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

[0041] This invention relates to the use of a human G protein-coupled receptor as a screening tool to identify agonists or antagonists of the aequorin luminescence resulting from expression of this receptor.

Example 1: Cloning of human GPCRx11 receptor

[0042] In order to identify and clone novel human GPCR (G-protein coupled receptor) the following approach was used. Sequences of the following GPCR: GPR8, ChemR23, HM74 and GPR14 were used as queries to search for homologies in public high-throughput genomic sequence databases (NCBI).

[0043] Using the above strategies, a novel human sequence of GPCR was identified. We called this new GPCR: GPCRx11 (SEQ ID number 1 and 2).

[0044] In order to clone the GPCRx11 sequence we performed a polymerase chain reaction (PCR) on total human genomic DNA. Primers were synthetized based upon the GPCRx11 human sequence and were as follows:

SEQ ID 23 GPCRx11 fw: 5'-ccggaattcaccatggatccaaccaccccg-3'
SEQ ID 24 GPCRx11 rv: 5'-ctagtctagactctacaccagactgcttctc-3'

[0045] Amplification resulted in a fragments of 0.99 kilobase containing the entire coding sequence of the GPCRx11 gene. This fragment was subcloned into the pCDNA3 (Invitrogen) vector for DNA sequencing analysis.

[0046] Nucleotide and deduced amino acid sequence of human GPCRx11 (SEQ ID NO 1)

```
          1  M   D   P   T   T   P   A   W   G   T   E   S   T   T   V
15
          1 ATG GAT CCA ACC ACC CCG GCC TGG GGA ACA GAA AGT ACA ACA GTG
45

         16  N   G   N   D   Q   A   L   L   L   L   C   G   K   E   T
30
         46 AAT GGA AAT GAC CAA GCC CTT CTT CTG CTT TGT GGC AAG GAG ACC
90

         31  L   I   P   V   F   L   I   L   F   I   A   L   V   G   L
45
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136 GTA GGA AAC GGG TTT GTG CTC TGG CTC CTG GGC TTC CGC ATG CGC
180

61 R N A F S V Y V L S L A G A D
75
181 AGG AAC GCC TTC TCT GTC TAC GTC CTC AGC CTG GCC GGG GCC GAC
225

76 F L F L C F Q I I N C L V Y L
90
226 TTC CTC TTC CTC TGC TTC CAG ATT ATA AAT TGC CTG GTG TAC CTC
270

91 S N F F C S I S I N F P S F F
105
271 AGT AAC TTC TTC TGT TCC ATC TCC ATC AAT TTC CCT AGC TTC TTC
315

106 T T V M T C A Y L A G L S M L
120
316 ACC ACT GTG ATG ACC TGT GCC TAC CTT GCA GGC CTG AGC ATG CTG
360

121 S T V S T E R C L S V L W P I
135
361 AGC ACC GTC AGC ACC GAG CGC TGC CTG TCC GTC CTG TGG CCC ATC
405

136 W Y R C R R P R H L S A V V C
150
406 TGG TAT CGC TGC CGC CGC CCC AGA CAC CTG TCA GCG GTC GTG TGT
450

151 V L L W A L S L L L S I L E G
165
451 GTC CTG CTC TGG GCC CTG TCC CTA CTG CTG AGC ATC TTG GAA GGG
495

166 K F C G F L F S D G D S G W C
180
496 AAG TTC TGT GGC TTC TTA TTT AGT GAT GGT GAC TCT GGT TGG TGT
540

181 Q T F D F I T A A W L I F L F
195
541 CAG ACA TTT GAT TTC ATC ACT GCA GCG TGG CTG ATT TTT TTA TTC
585

196 M V L C G S S L A L L V R I L
210
586 ATG GTT CTC TGT GGG TCC AGT CTG GCC CTG CTG GTC AGG ATC CTC
630

211 C G S R G L P L T R L Y L T I
225

240

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      676 CTG CTC ACA GTG CTG GTG TTC CTC CTC TGC GGC CTG CCC TTT GGC
  720

      241  I   Q   W   F   L   I   L   W   I   W   K   D   S   D   V
5 255
      721 ATT CAG TGG TTC CTA ATA TTA TGG ATC TGG AAG GAT TCT GAT GTC
  765

      256  L   F   C   H   I   H   P   V   S   V   V   L   S   S   L
10 270
      766 TTA TTT TGT CAT ATT CAT CCA GTT TCA GTT GTC CTG TCA TCT CTT
  810

      271  N   S   S   A   N   P   I   I   Y   F   F   V   G   S   F
15 285
      811 AAC AGC AGT GCC AAC CCC ATC ATT TAC TTC TTC GTG GGC TCT TTT
  855

      286  R   K   Q   W   R   L   Q   Q   P   I   L   K   L   A   L
20 300
      856 AGG AAG CAG TGG CGG CTG CAG CAG CCG ATC CTC AAG CTG GCT CTC
  900

      301  Q   R   A   L   Q   D   I   A   E   V   D   H   S   E   G
25 315
      901 CAG AGG GCT CTG CAG GAC ATT GCT GAG GTG GAT CAC AGT GAA GGA
  945

      316  C   F   R   Q   G   T   P   E   M   S   R   S   S   L   V
30 330
      946 TGC TTC CGT CAG GGC ACC CCG GAG ATG TCG AGA AGC AGT CTG GTG
  990

      331  *
35 331
      991 TAG
  993
```

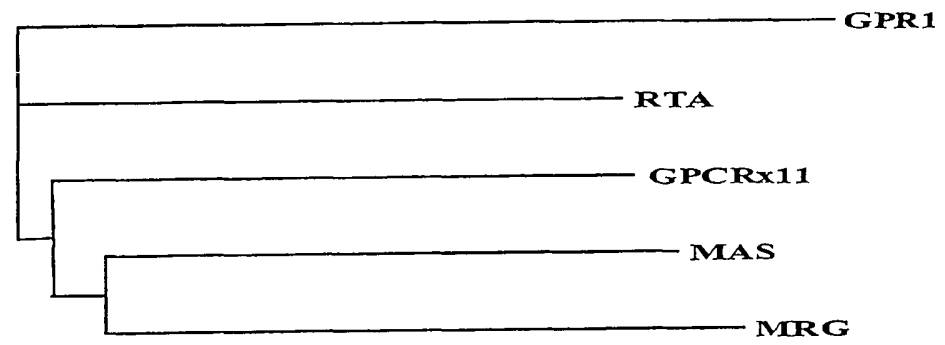
40 **[0047]** Amino acid sequence of human GPCRx11 (330 amino acids) (SEQ ID NO:2). The seven predicted transmembrane domaines are underlined.

MDPTTPAWGTESTTVNGNDQALLLLCGKETLIPVFLILFIALVGLVGNGFVLWLLGFRM
45 RRNAFSVYVLSLAGADFLFLCFQIINCLVYLSNFFCSISINFPSFFTTVMTCAYLAGLS
MLSTVSTERCLSVLWPIWYRCRRPRHLSAVVCVLLWALSLLLSILEGKFCGFLFSDGDS
GWCQTFDFITAAWLIFLFMVLCGSSLALLVRILCGSRGLPLTRLYLTILLTVLVFLLCG
LPFGIQWFLILWIWKDSDVLFCHIHPVSVVLSSLNSSANPIIYFFVGSFRKQWRLQQPI

[0048] At the amino acid sequence level, the human GPCRx11 is 37% identical to the rat RTA receptor. The gene coding GPCRx11 is located on chromosome 11.

Alignment of GPCRx11 (fig.1)

[0049] Alignment of the amino acid sequence of GPCRx11 with RTA and other RTA related sequences were performed using ClustalX algorithm. Then, the dendrogram was constucted using TreeView algorithm.



Tissular distribution of GPCRx11

[0050] Reverse transcription-polymerase chain reaction (RT-PCR) experiments were carried out using a panel of polyA$^+$ RNA (Clontech). The primers were as follows: GPCRx11 sense primer (SEQ ID NO 25: 5'-TTCTCTGTCTACGTCCTCAG-3') and GPCRx11 antisense primer (SEQ ID NO 26: 5'-GTCCTGTCATCTCTTAACAG-3'). The expected size of the amplified DNA band was 586 bp. Approximately 75 ng of poly A$^+$ RNA was reverse transcribed with superscript II (Life Technologies) and used for PCR. PCR was performed under the following conditions: denaturation at 94°C for 3 min, 38 cycles at 94°C for 1 min, 58°C for 2 min and 72°C

[0051] GPCRx11 mRNA was assayed by RT-PCR in 16 human tissues. A strong band of expected size (586 bp) was detected in testis, at lower levels in uterus and thymus, while not in pituitary gland, spinal cord, pancreas, small intestine, placenta, stomach, liver, lung, spleen, brain, heart, kidney and skeletal muscle.

Functional assay for GPCRx11

[0052] GPCRx11 expressing clones have been obtained by transfection of CHO-K1 cells coexpressing mitochondrial apoaequorin and Galpha16, limit dilution and selection by northern blotting. Positive clones were used for screening with a reference peptidic library containing 250 peptides and neuropeptides at a concentration of 100 nM. A specific activity was obtained with angiopeptin (D-NaI-Cys-Tyr-D-trp-Lys-Val-Cys-Thr-NH2 with a disulfide bridge between the two cysteines) and confirmed by a dose respone curve (see figure 1). Additional related peptides were tested using the same cells. Amongst the different peptides tested, somatostatin analog (D2-NaI-Cys-Tyr-D-trp-Lys-Val-Cys-D2-NaI-NH2) exhibited similar affinity. Somatostatin 14 has no activity on GPCRx11.

Material. All chemicals were obtained from Sigma, unless stated. The cell culture media were from Gibco BRL and the peptides from bachem

Aequorin assays. CHO-K1 cell lines expressing GPCRx11 receptors, $Galpha_{16}$ and mitochondrial apoaequorin were established. A functional assay based on the luminescence of mitochondrial aequorin following intracellular Ca^{2+} release (1) was [illegible]

[illegible] medium, incubated with 5 μM Coelenterazine H (Molecular

Probes) for 4 hours at room temperature. Cells were then washed in DMEM-F12 medium and resuspended at a concentration of 0.5 X 10^6 cells/ml. Cells were then mixed with the peptides and the light emission recorded during 30 sec. using a Microlumat luminometer (Perkin Elmer). Results are expressed as Relative Light Units (RLU).

Antibodies

[0053] Antibodies directed against GPCRx11 have been produced by repeated injections of plasmid encoding GPCRx11 to mice. Serum has been collected following 5 injections and used for flow cytometry analysis with cells transfected with GPCRx11. Several sera were positive and can be used for immunohistochemistry and other related applications

Example 2 : Cloning of the other sequences related to G-protein-coupled receptors

[0054] In order to identify and clone novel human DNA sequences related to GPCR, the following approche was used. Sequences of the following GPCR: GPR8, ChemR23, HM74 and GPR14 were used as queries to search for homologies in public high-throughput genomic sequence databases (NCBI).

[0055] Using the above strategies, ten novel human sequences of GPCR were identified. None of these clones contain introns :

GPCRx2, SEQ ID NO 3

GPCRx5, SEQ ID NO 5

GPCRx7, SEQ ID NO 7

GPCRx9, SEQ ID NO 9

GPCRx14, SEQ ID NO 11

GPCRx18, SEQ ID NO 17

GPCRx19, SEQ ID NO 19

GPCRx20, SEQ ID NO 21

[0056] In order to clone these GPCRx sequences, a polymerase chain reaction (PCR) was performed on total human genomic DNA. Primers were synthetized based upon the human sequences described above and were as follows:

SEQ ID NO 27 GPCRx2 fw: 5'-ccggaattcaccatggagtcctcacccatc-3'
SEQ ID NO 28 GPCRx2 rv: 5'-ctagtctagacatcatgactccagccggg-3'

SEQ ID NO 29 GPCRx5 fw: 5'-ccggaattcaccatggatccaaccatctcaacc-3'
SEQ ID NO 30 GPCRx5 rv: 5'-ctagtctagatcactgctccaatctgcttc-3'

SEQ ID NO 31 GPCRx7 fw:
5'-ccggaattcaccatgaaccagactttgaatagcagtgg-3'
SEQ ID NO 32 GPCRx7 rv:
5'-ctagtctagatctcaagcccccatctcattggtgccc-3'

SEQ ID NO 33 GPCRx9 fw: 5'-ccggaattcaccatggaagctgacctgg-3'
SEQ ID NO 34 GPCRx9 rv: 5'-ctagtctagactcacgtggggcctgcgcc-3'

SEQ ID NO 35 GPCRx14 fw: 5'-ccggaattcgccatgtacaacgggtcg-3'
SEQ ID NO 36 GPCRx14 rv: 5'-ctagtctagattcagtgccactcaacaatg-3'

[0057] Amplification resulted in a fragments of approximately 1 - 1.5 kilobase containing the entire coding sequence of the human genes. These fragments obtained were subcloned into the pCDNA3 (Invitrogen) vector for DNA sequencing analysis.

Tissue distribution of identified (GPCRx) receptors

[0058] To determine the tissue distribution of different

isolated from human tissues (Clontech). The oligo(dT)

primer was used in the reverse transcription step. Then, different GPCRx cDNA were amplified with specifics primers.

	GPCRx 2	GPCRx 7	GPCRx 9	GPCRx 14	GPCRx 16	GPCRx 17	GPCRx 18	GPCRx 19	GPCRx 20
Li	-	-	-	-	-	-	-	-	+
Lu	+/-	-	+	+	-	++	-	-	++
Sp	-	-	++	+	-	-	-	-	+
Te	-	+	-	++	-	++	-	+/-	+
Br	++	-	-	-	-	-	++	-	++
He	-	-	-	-	-	-	-	-	++
Ki	+/-	-	-	+	-	++	-	-	+
Sk.m	-	-	-	-	-	+	-	-	++
Pi.G	-	-	-	-	-	-	++	+/-	+
Sp.C	++	-	-	-	-	++	+/-	+/-	+/-
Th	+/-	-	+	-	-	++	-	-	++
Pa	-	-	-	-	-	++	+/-	-	-
S.In	+/-	-	+	-	-	++	-	-	+
Ut	-	-	-	-	-	++	-	+/-	+
Pl	-	-	-	++	++	-	-	-	+
St	-	-	+	+/-	-	++	-	-	+

Table 1: Tissue distribution of GPCRxs: The presence or absence of differents GPCRx was determined by RT-PCR analysis. ++, strong signal; +, signal clearly detected; +/-, weak signal; -, signal not detected. The tissues are the following: Li, liver; Lu, lung; Sp, Spleen; Te, testis; Br, Brain; He, Heart; Ki, Kidney; Sk.M, Skeletal muscle; Pi.G, Pituitary gland; Sp.C, spinal cord; Th, Thymus; Pa,

Reference

1. Stables, J., A. Green, F. Marshall, N. Fraser, E. Knight, M. Sautel, G. Milligan, M. Lee, and S. Rees. 1997. A bioluminescent assay for agonist activity at potentially any G-protein-coupled receptor. *Anal. Biochem.* 252:115-126.

2. Blanpain, C., I. Migeotte, B. Lee, J. Vakili, B.J. Doranz, C. Govaerts, G. Vassart, R.W. Doms, and M. Parmentier. 1999 CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. *Blood* 94:1899-1905.

Nucleotide and deduced amino acid sequence of human GPCRx2 (SEQ ID NO: 3 and 4 respectively)

```
   1  M   E   S   S   P   I   P   Q   S   S   G   N   S   S   T      15
   1 ATG GAG TCC TCA CCC ATC CCC CAG TCA TCA GGG AAC TCT TCC ACT      45

  16  L   G   R   V   P   Q   T   P   G   P   S   T   A   S   G      30
  46 TTG GGG AGG GTC CCT CAA ACC CCA GGT CCC TCT ACT GCC AGT GGG      90

  31  V   P   E   V   G   L   R   D   V   A   S   E   S   V   A      45
  91 GTC CCG GAG GTG GGG CTA CGG GAT GTT GCT TCG GAA TCT GTG GCC     135

  46  L   F   F   M   L   L   L   D   L   T   A   V   A   G   N      60
 136 CTC TTC TTC ATG CTC CTG CTG GAC TTG ACT GCT GTG GCT GGC AAT     180

  61  A   A   V   M   A   V   I   A   K   T   P   A   L   R   K      75
 181 GCC GCT GTG ATG GCC GTG ATC GCC AAG ACG CCT GCC CTC CGA AAA     225

  76  F   V   F   V   F   H   L   C   L   V   D   L   L   A   A      90
 226 TTT GTC TTC GTC TTC CAC CTC TGC CTG GTG GAC CTG CTG GCT GCC     270

  91  L   T   L   M   P   L   A   M   L   S   S   S   A   L   F     105
 271 CTG ACC CTC ATG CCC CTG GCC ATG CTC TCC AGC TCT GCC CTC TTT     315

 106  D   H   A   L   F   G   E   V   A   C   R   L   Y   L   F     120
 316 GAC CAC GCC CTC TTT GGG GAG GTG GCC TGC CGC CTC TAC TTG TTT     360

 121  L   S   V   C   F   V   S   L   A   I   L   S   V   S   A     135
 361 CTG AGC GTG TGC TTT GTC AGC CTG GCC ATC CTC TCG GTG TCA GCC     405

 136  I   N   V   E   R   Y   Y   Y   V   V   H   P   M   R   Y     150
 406 ATC AAT GTG GAG CGC TAC TAT TAC GTA GTC CAC CCC ATG CGC TAC     450

 151  E   V   R   M   T   L   G   L   V   A   S   V   L   V   G     165
 451 GAG GTG CGC ATG ACG CTG GGG CTG GTG GCC TCT GTG CTG GTG GGT     495

 166  V   W   V   K   A   L   A   M   A   S   V   P   V   L   G     180
 496 GTG TGG GTG AAG GCC TTG GCC ATG GCT TCT GTG CCA GTG TTG GGA     540

 181  R   V   S   W   E   E   G   A   P   S   V   P   P   G   C     195
 541 AGG GTC TCC TGG GAG GAA GGA GCT CCC AGT GTC CCC CCA GGC TGT     585

 196  S   L   Q   W   S   H   S   A   Y   C   Q   L   F   V   V     210
 586 TCA CTC CAG TGG AGC CAC AGT GCC TAC TGC CAG CTT TTT GTG GTG     630

 211  V   F   A   V   L   Y   F   L   L   P   L   L   L   I   L     225
 631 GTC TTT GCT GTC CTT TAC TTT CTG TTG CCC CTG CTC CTC ATA CTT     675

 226  V   V   Y   C   S   M   F   R   V   A   R   V   A   A   M     240
 676 GTG GTC TAC TGC AGC ATG TTC CGA GTG GCC CGC GTG GCT GCC ATG     720

 241  Q   H   G   P   L   P   T   W   M   E   T   P   R   Q   R     255
 721 CAG CAC GGG CCG CTG CCC ACG TGG ATG GAG ACA CCC CGG CAA CGC     765
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271  A   P   Q   T   T   P   H   R   T   F   G   G   G   K   A      285
811  GCC CCC CAG ACC ACC CCA CAC CGG ACG TTT GGG GGA GGG AAA GCA    855

286  A   V   V   L   L   A   V   G   G   Q   F   L   L   C   W      300
856  GCA GTG GTT CTC CTG GCT GTG GGG GGA CAG TTC CTG CTC TGT TGG    900

301  L   P   Y   F   S   F   H   L   Y   V   A   L   S   A   Q      315
901  TTG CCC TAC TTC TCT TTC CAC CTC TAT GTT GCC CTG AGT GCT CAG    945

316  P   I   S   T   G   Q   V   E   S   V   V   T   W   I   G      330
946  CCC ATT TCA ACT GGG CAG GTG GAG AGT GTG GTC ACC TGG ATT GGC    990

331  Y   F   C   F   T   S   N   P   F   F   Y   G   C   L   N      345
991  TAC TTT TGC TTC ACT TCC AAC CCT TTC TTC TAT GGA TGT CTC AAC   1035

346  R   Q   I   R   G   E   L   S   K   Q   F   V   C   F   F      360
1036 CGG CAG ATC CGG GGG GAG CTC AGC AAG CAG TTT GTC TGC TTC TTC   1080

361  K   P   A   P   E   E   E   L   R   L   P   S   R   E   G      375
1081 AAG CCA GCT CCA GAG GAG GAG CTG AGG CTG CCT AGC CGG GAG GGC   1125

376  S   I   E   E   N   F   L   Q   F   L   Q   G   T   G   C      390
1126 TCC ATT GAG GAG AAC TTC CTG CAG TTC CTT CAG GGG ACT GGC TGT   1170

391  P   S   E   S   W   V   S   R   P   L   P   S   P   K   Q      405
1171 CCT TCT GAG TCC TGG GTT TCC CGA CCC CTA CCC AGC CCC AAG CAG   1215

406  E   P   P   A   V   D   F   R   I   P   G   Q   I   A   E      420
1216 GAG CCA CCT GCT GTT GAC TTT CGA ATC CCA GGC CAG ATA GCT GAG   1260

421  E   T   S   E   F   L   E   Q   Q   L   T   S   D   I   I      435
1261 GAG ACC TCT GAG TTC CTG GAG CAG CAA CTC ACC AGC GAC ATC ATC   1305

436  M   S   D   S   Y   L   R   P   A   A   S   P   R   L   E      450
1306 ATG TCA GAC AGC TAC CTC CGT CCT GCC GCC TCA CCC CGG CTG GAG   1350

451  S   *                                                          452
1351 TCA TGA                                                       1356
```

Amino acid sequence of human GPCRx2 (451 amino acids) (SEQ ID NO: 4). The seven predicted transmembrane domaines are underlined.

MESSPIPQSSGNSSTLGRVPQTPGPSTASGVPEVGLRDVASESVALFFMLLLDLTAVAGNAAVMAVIAKTPALRKFVFVF
HLCLVDLLAALTLMPLAMLSSSALFDHALFGEVACRLYLFLSVCFVSLAILSVSAINVERYYYVVHPMRYEVRMTLGLVA
SVLVGVWVKALAMASVPVLGRVSWEEGAPSVPPGCSLQWSHSAYCQLFVVVFAVLYFLLPLLLILVVYCSMFRVARVAAM
QHGPLPTWMETPRQRSESLSSRSTMVTSSGAPQTTPHRTFGGGKAAVVLLAVGGQFLLCWLPYFSFHLYVALSAQPISTG
QVESVVTWIGYFCFTSNPFFYGCLNRQIRGELSKQFVCFFKPAPEEELRLPSREGSIEENFLQFLQGTGCPSESWVSRPL
PSPKQEPPAVDFRIPGQIAEETSEFLEQQLTSDIIMSDSYLRPAASPRLES

At the amino acid sequence level, the human GPCRx2 is 23% identical to the human histamine H2 receptor.

Nucleotide and deduced amino acid sequence of human GPCRx5 (SEQ ID NO: 5 and 6 respectively)

```
   1  M   D   P   T   I   S   T   L   D   T   E   L   T   P   I      15
   1 ATG GAT CCA ACC ATC TCA ACC TTG GAC ACA GAA CTG ACA CCA ATC      45

  16  N   G   T   E   E   T   L   C   Y   K   Q   T   L   S   L      30
  46 AAC GGA ACT GAG GAG ACT CTT TGC TAC AAG CAG ACC TTG AGC CTC      90

  31  T   V   L   T   C   I   V   S   L   V   G   L   T   G   N      45
  91 ACG GTG CTG ACG TGC ATC GTT TCC CTT GTC GGG CTG ACA GGA AAC     135

  46  A   V   V   L   W   L   L   G   C   R   M   R   R   N   A      60
 136 GCA GTT GTG CTC TGG CTC CTG GGC TGC CGC ATG CGC AGG AAC GCC     180

  61  F   S   I   Y   I   L   N   L   A   A   A   D   F   L   F      75
 181 TTC TCC ATC TAC ATC CTC AAC TTG GCC GCA GCA GAC TTC CTC TTC     225

  76  L   S   G   R   L   I   Y   S   L   L   S   F   I   S   I      90
 226 CTC AGC GGC CGC CTT ATA TAT TCC CTG TTA AGC TTC ATC AGT ATC     270

  91  P   H   T   I   S   K   I   L   Y   P   V   M   M   F   S     105
 271 CCC CAT ACC ATC TCT AAA ATC CTC TAT CCT GTG ATG ATG TTT TCC     315

 106  Y   F   A   G   L   S   F   L   S   A   V   S   T   E   R     120
 316 TAC TTT GCA GGC CTG AGC TTT CTG AGT GCC GTG AGC ACC GAG CGC     360

 121  C   L   S   V   L   W   P   I   W   Y   R   C   H   R   P     135
 361 TGC CTG TCC GTC CTG TGG CCC ATC TGG TAC CGC TGC CAC CGC CCC     405

 136  T   H   L   S   A   V   V   C   V   L   L   W   A   L   S     150
 406 ACA CAC CTG TCA GCG GTG GTG TGT GTC CTG CTC TGG GCC CTG TCC     450

 151  L   L   R   S   I   L   E   W   M   L   C   G   F   L   F     165
 451 CTG CTG CGG AGC ATC CTG GAG TGG ATG TTA TGT GGC TTC CTG TTC     495

 166  S   G   A   D   S   A   W   C   Q   T   S   D   F   I   T     180
 496 AGT GGT GCT GAT TCT GCT TGG TGT CAA ACA TCA GAT TTC ATC ACA     540

 181  V   A   W   L   I   F   L   C   V   V   L   C   G   S   S     195
 541 GTC GCG TGG CTG ATT TTT TTA TGT GTG GTT CTC TGT GGG TCC AGC     585

 196  L   V   L   L   I   R   I   L   C   G   S   R   K   I   P     210
 586 CTG GTC CTG CTG ATC AGG ATT CTC TGT GGA TCC CGG AAG ATA CCG     630

 211  L   T   R   L   Y   V   T   I   L   L   T   V   L   V   F     225
 631 CTG ACC AGG CTG TAC GTG ACC ATC CTG CTC ACA GTA CTG GTC TTC     675

 226  L   L   C   G   L   P   F   G   I   Q   F   F   L   F   L     240
 676 CTC CTC TGT GGC CTG CCC TTT GGC ATT CAG TTT TTC CTA TTT TTA     720

 241  W   I   H   V   D   R   E   V   L   F   C   H   V   H   L     255
 721 TGG ATC CAC GTG GAC AGG GAA GTC TTA TTT TGT CAT GTT CAT CTA     765

 256  V   S   I   F   L   S   A   L   N   S   S   A   N   P   I     270
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271  I   Y   F   F   V   G   S   F   R   Q   R   Q   N   R   Q      285
811 ATT TAC TTC TTC GTG GGC TCC TTT AGG CAG CGT CAA AAT AGG CAG     855

286  N   L   K   L   V   L   Q   R   A   L   Q   D   A   S   E      300
856 AAC CTG AAG CTG GTT CTC CAG AGG GCT CTG CAG GAC GCG TCT GAG     900

301  V   D   E   G   G   G   Q   L   P   E   E   I   L   E   L      315
901 GTG GAT GAA GGT GGA GGG CAG CTT CCT GAG GAA ATC CTG GAG CTG     945

316  S   G   S   R   L   E   Q   *                                  323
946 TCG GGA AGC AGA TTG GAG CAG TGA                                 969
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Amino acid sequence of human GPCRx5 (322 amino acids) (SEQ ID NO:6). The seven predicted transmembrane domaines are underlined.

MDPTISTLDTELTPINGTEETLCYKQTLSLTVLTCIVSLVGLTGNAVVLWLLGCRMRRNAFSIYILNLAAADFLFLSGRL
IYSLLSFISIPHTISKILYPVMMFSYFAGLSFLSAVSTERCLSVLWPIWYRCHRPTHLSAVVCVLLWALSLLRSILEWML
CGFLFSGADSAWCQTSDFITVAWLIFLCVVLCGSSLVLLIRILCGSRKIPLTRLYVTILLTVLVFLLCGLPFGIQFFLFL
WIHVDREVLFCHVHLVSIFLSALNSSANPIIYFFVGSFRQRQNRQNLKLVLQRALQDASEVDEGGGQLPEEILELSGSRL
EQ

At the amino acid sequence level, the human GPCRx5 is 31% identical to the human mas receptor.

Nucleotide and deduced amino acid sequence of human GPCRx7 (SEQ ID NO: 7 and 8 respectively)

```
   1 M   N   Q   T   L   N   S   S   G   T   V   E   S   A   L      15
   1 ATG AAC CAG ACT TTG AAT AGC AGT GGG ACC GTG GAG TCA GCC CTA      45

  16 N   Y   S   R   G   S   T   V   H   T   A   Y   L   V   L      30
  46 AAC TAT TCC AGA GGG AGC ACA GTG CAC ACG GCC TAC CTG GTG CTG      90

  31 S   S   L   A   M   F   T   C   L   C   G   M   A   G   N      45
  91 AGC TCC CTG GCC ATG TTC ACC TGC CTG TGC GGG ATG GCA GGC AAC     135

  46 S   M   V   I   W   L   L   G   F   R   M   H   R   N   P      60
 136 AGC ATG GTG ATC TGG CTG CTG GGC TTT CGA ATG CAC AGG AAC CCC     180

  61 F   C   I   Y   I   L   N   L   A   A   A   D   L   L   F      75
 181 TTC TGC ATC TAT ATC CTC AAC CTG GCG GCA GCC GAC CTC CTC TTC     225

  76 L   F   S   M   A   S   T   L   S   L   E   T   Q   P   L      90
 226 CTC TTC AGC ATG GCT TCC ACG CTC AGC CTG GAA ACC CAG CCC CTG     270

  91 V   N   T   T   D   K   V   H   E   L   M   K   R   L   M     105
 271 GTC AAT ACC ACT GAC AAG GTC CAC GAG CTG ATG AAG AGA CTG ATG     315

 106 Y   F   A   Y   T   V   G   L   S   L   L   T   A   I   S     120
 316 TAC TTT GCC TAC ACA GTG GGC CTG AGC CTG CTG ACG GCC ATC AGC     360

 121 T   Q   R   C   L   S   V   L   F   P   I   W   F   K   C     135
 361 ACC CAG CGC TGT CTC TCT GTC CTC TTC CCT ATC TGG TTC AAG TGT     405

 136 H   R   P   R   H   L   S   A   W   V   C   G   L   L   W     150
 406 CAC CGG CCC AGG CAC CTG TCA GCC TGG GTG TGT GGC CTG CTG TGG     450

 151 T   L   C   L   L   M   N   G   L   T   S   S   F   C   S     165
 451 ACA CTC TGT CTC CTG ATG AAC GGG TTG ACC TCT TCC TTC TGC AGC     495

 166 K   F   L   K   F   N   E   D   R   C   F   R   V   D   M     180
 496 AAG TTC TTG AAA TTC AAT GAA GAT CGG TGC TTC AGG GTG GAC ATG     540

 181 V   Q   A   A   L   I   M   G   V   L   T   P   V   M   T     195
 541 GTC CAG GCC GCC CTC ATC ATG GGG GTC TTA ACC CCA GTG ATG ACT     585

 196 L   S   S   L   T   L   F   V   W   V   R   R   S   S   Q     210
 586 CTG TCC AGC CTG ACC CTC TTT GTC TGG GTG CGG AGG AGC TCC CAG     630

 211 Q   W   R   R   Q   P   T   R   L   F   V   V   V   L   A     225
 631 CAG TGG CGG CGG CAG CCC ACA CGG CTG TTC GTG GTG GTC CTG GCC     675

 226 S   V   L   V   F   L   I   C   S   L   P   L   S   I   Y     240
 676 TCT GTC CTG GTG TTC CTC ATC TGT TCC CTG CCT CTG AGC ATC TAC     720

 241 W   F   V   L   Y   W   L   S   L   P   P   E   M   Q   V     255
 721 TGG TTT GTG CTC TAC TGG TTG AGC CTG CCG CCC GAG ATG CAG GTC     765
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271  A   N   P   V   I   Y   F   L   V   G   S   R   R   S   H      285
811  GCC AAC CCC GTC ATC TAC TTC CTG GTG GGC AGC CGG AGG AGC CAC     855

286  R   L   P   T   R   S   L   G   T   V   L   Q   Q   A   L      300
856  AGG CTG CCC ACC AGG TCC CTG GGG ACT GTG CTC CAA CAG GCG CTT     900

301  R   E   E   P   E   L   E   G   G   E   T   P   T   V   G      315
901  CGC GAG GAG CCC GAG CTG GAA GGT GGG GAG ACG CCC ACC GTG GGC     945

316  T   N   E   M   G   A   *                                      322
946  ACC AAT GAG ATG GGG GCT TGA                                     966
```

Amino acid sequence of human GPCRx7 (321 amino acids) (SEQ ID NO:8). The seven predicted transmembrane domaines are underlined.

MNQTLNSSGTVESALNYSRGSTVHTAYLVLSSLAMFTCLCGMAGNSMVIWLLGFRMHRNPFCIYILNLAAADLLFLFSMA
STLSLETQPLVNTTDKVHELMKRLMYFAYTVGLSLLTAISTQRCLSVLFPIWfKCHRPRHLSAWVCGLLWTLCLLMNGLT
SSFCSKFLKFNEDRCFRVDMVQAALIMGVLTPVMTLSSLTLFVWVRRSSQQWRRQPTRLFVVVLASVLVFLICSLPLSIY
WFVLYWLSLPPEMQVLCFSLSRLSSSVSSSANPVIYFLVGSRRSHRLPTRSLGTVLQQALREEPELEGGETPTVGTNEMG
A

At the amino acid sequence level, the human GPCRx7 is 29% identical to the rat RTA receptor.

Nucleotide and deduced amino acid sequence of human GPCRx9 (SEQ ID NO: 9 and 10 respectively)

```
   1  M   E   A   D   L   G   A   T   G   H   R   P   R   T   E      15
   1 ATG GAA GCT GAC CTG GGT GCC ACT GGC CAC AGG CCC CGC ACA GAG      45

  16  L   D   D   E   D   S   Y   P   Q   G   G   W   D   T   V      30
  46 CTT GAT GAT GAG GAC TCC TAC CCC CAA GGT GGC TGG GAC ACG GTC      90

  31  F   L   V   A   L   L   L   L   G   L   P   A   N   G   L      45
  91 TTC CTG GTG GCC CTG CTG CTC CTT GGG CTG CCA GCC AAT GGG TTG     135

  46  M   A   W   L   A   G   S   Q   A   R   H   G   A   G   T      60
 136 ATG GCG TGG CTG GCC GGC TCC CAG GCC CGG CAT GGA GCT GGC ACG     180

  61  R   L   A   L   L   L   L   S   L   A   L   S   D   F   L      75
 181 CGT CTG GCG CTG CTC CTG CTC AGC CTG GCC CTC TCT GAC TTC TTG     225

  76  F   L   A   A   A   A   F   Q   I   L   E   I   R   H   G      90
 226 TTC CTG GCA GCA GCG GCC TTC CAG ATC CTA GAG ATC CGG CAT GGG     270

  91  G   H   W   P   L   G   T   A   A   C   R   F   Y   Y   F     105
 271 GGA CAC TGG CCG CTG GGG ACA GCT GCC TGC CGC TTC TAC TAC TTC     315

 106  L   W   G   V   S   Y   S   S   G   L   F   L   L   A   A     120
 316 CTA TGG GGC GTG TCC TAC TCC TCC GGC CTC TTC CTG CTG GCC GCC     360

 121  L   S   L   D   R   C   L   L   A   L   C   P   H   W   Y     135
 361 CTC AGC CTC GAC CGC TGC CTG CTG GCG CTG TGC CCA CAC TGG TAC     405

 136  P   G   H   R   P   V   R   L   P   L   W   V   C   A   G     150
 406 CCT GGG CAC CGC CCA GTC CGC CTG CCC CTC TGG GTC TGC GCC GGT     450

 151  V   W   V   L   A   T   L   F   S   V   P   W   L   V   F     165
 451 GTC TGG GTG CTG GCC ACA CTC TTC AGC GTG CCC TGG CTG GTC TTC     495

 166  P   E   A   A   V   W   W   Y   D   L   V   I   C   L   D     180
 496 CCC GAG GCT GCC GTC TGG TGG TAC GAC CTG GTC ATC TGC CTG GAC     540

 181  F   W   D   S   E   E   L   S   L   R   M   L   E   V   L     195
 541 TTC TGG GAC AGC GAG GAG CTG TCG CTG AGG ATG CTG GAG GTC CTG     585

 196  G   G   F   L   P   F   L   L   L   L   V   C   H   V   L     210
 586 GGG GGC TTC CTG CCT TTC CTC CTG CTG CTC GTC TGC CAC GTG CTC     630

 211  T   Q   A   T   A   C   R   T   C   H   R   Q   Q   Q   P     225
 631 ACC CAG GCC ACA GCC TGT CGC ACC TGC CAC CGC CAA CAG CAG CCC     675

 226  A   A   C   R   G   F   A   R   V   A   R   T   I   L   S     240
 676 GCA GCC TGC CGG GGC TTC GCC CGT GTG GCC AGG ACC ATT CTG TCA     720

 241  A   Y   V   V   L   R   L   P   Y   Q   L   A   Q   L   L     255
 721 GCC TAT GTG GTC CTG AGG CTG CCC TAC CAG CTG GCC CAG CTG CTC     765

 256  Y   L   A   F   L   W   D   V   Y   S   G   Y   L   L   W     270
```

```
 271  E   A   L   V   Y   S   D   Y   L   I   L   L   N   S   C      285
 811 GAG GCC CTG GTC TAC TCC GAC TAC CTG ATC CTA CTC AAC AGC TGC     855

 286  L   S   P   F   L   C   L   M   A   S   A   D   L   R   T      300
 856 CTC AGC CCC TTC CTC TGC CTC ATG GCC AGT GCC GAC CTC CGG ACC     900

 301  L   L   R   S   V   L   S   S   F   A   A   A   L   C   E      315
 901 CTG CTG CGC TCC GTG CTC TCG TCC TTC GCG GCA GCT CTC TGC GAG     945

 316  E   R   P   G   S   F   T   P   T   E   P   Q   T   Q   L      330
 946 GAG CGG CCG GGC AGC TTC ACG CCC ACT GAG CCA CAG ACC CAG CTA     990

 331  D   S   E   G   P   T   L   P   E   P   M   A   E   A   Q      345
 991 GAT TCT GAG GGT CCA ACT CTG CCA GAG CCG ATG GCA GAG GCC CAG    1035

 346  S   Q   M   D   P   V   A   Q   P   Q   V   N   P   T   L      360
1036 TCA CAG ATG GAT CCT GTG GCC CAG CCT CAG GTG AAC CCC ACA CTC    1080

 361  Q   P   R   S   D   P   T   A   Q   P   Q   L   N   P   T      375
1081 CAG CCA CGA TCG GAT CCC ACA GCT CAG CCA CAG CTG AAC CCT ACG    1125

 376  A   Q   P   Q   S   D   P   T   A   Q   P   Q   L   N   L      390
1126 GCC CAG CCA CAG TCG GAT CCC ACA GCC CAG CCA CAG CTG AAC CTC    1170

 391  M   A   Q   P   Q   S   D   S   V   A   Q   P   Q   A   D      405
1171 ATG GCC CAG CCA CAG TCA GAT TCT GTG GCC CAG CCA CAG GCA GAC    1215

 406  T   N   V   Q   T   P   A   P   A   A   S   S   V   P   S      420
1216 ACT AAC GTC CAG ACC CCT GCA CCT GCT GCC AGT TCT GTG CCC AGT    1260

 421  P   C   D   E   A   S   P   T   P   S   S   H   P   T   P      435
1261 CCC TGT GAT GAA GCT TCC CCA ACC CCA TCC TCG CAT CCT ACC CCA    1305

 436  G   A   L   E   D   P   A   T   P   P   A   S   E   G   E      450
1306 GGG GCC CTT GAG GAC CCA GCC ACA CCT CCT GCC TCT GAA GGA GAA    1350

 451  S   P   S   S   T   P   P   E   A   A   P   G   A   G   P      465
1351 AGC CCC AGC AGC ACC CCG CCA GAG GCG GCC CCG GGC GCA GGC CCC    1395

 466  T   *                                                          467
1396 ACG TGA                                                        1401
```

Amino acid sequence of human GPCRx9 (466 amino acids) (SEQ ID NO:10). The six predicted transmembrane domaines are underlined.

MEADLGATGHRPRTELDDEDSYPQGGWDTVFLVALLLLGLPANGLMAWLAGSQARHGAGTRLALLLLSLALSDFLFLAAA
AFQILEIRHGGHWPLGTAACRFYYFLWGVSYSSGLFLLAALSLDRCLLALCPHWYPGHRPVRLPLWVCAGVWVLATLFSV
PWLVFPEAAVWWYDLVICLDFWDSEELSLRMLEVLGGFLPFLLLLVCHVLTQATACRTCHRQQQPAACRGFARVARTILS
AYVVLRLPYQLAQLLYLAFLWDVYSGYLLWEALVYSDYLILLNSCLSPFLCLMASADLRTLLRSVLSSFAAALCEERPGS
FTPTEPQTQLDSEGPTLPEPMAEAQSQMDPVAQPQVNPTLQPRSDPTAQPQLNPTAQPQSDPTAQPQLNLMAQPQSDSVA
QPQADTNVQTPAPAASSVPSPCDEASPTPSSHPTPGALEDPATPPASEGESPSSTPPEAAPGAGPT

At the amino acid sequence level, the human GPCRx9 is 33% identical to the human ChemR23 receptor.

Nucleotide and deduced amino acid sequence of human GPCRx14 (SEQ ID NO: 11 and 12 respectively)

```
   1  M   Y   N   G   S   C   C   R   I   E   G   D   T   I   S      15
   1 ATG TAC AAC GGG TCG TGC TGC CGC ATC GAG GGG GAC ACC ATC TCC      45

  16  Q   V   M   P   P   L   L   I   V   A   F   V   L   G   A      30
  46 CAG GTG ATG CCG CCG CTG CTC ATT GTG GCC TTT GTG CTG GGC GCA      90

  31  L   G   N   G   V   A   L   C   G   F   C   F   H   M   K      45
  91 CTA GGC AAT GGG GTC GCC CTG TGT GGT TTC TGC TTC CAC ATG AAG     135

  46  T   W   K   P   S   T   V   Y   L   F   N   L   A   V   A      60
 136 ACC TGG AAG CCC AGC ACT GTT TAC CTT TTC AAT TTG GCC GTG GCT     180

  61  D   F   L   L   M   I   C   L   P   F   R   T   D   Y   Y      75
 181 GAT TTC CTC CTT ATG ATC TGC CTG CCT TTT CGG ACA GAC TAT TAC     225

  76  L   R   R   R   H   W   A   F   G   D   I   P   C   R   V      90
 226 CTC AGA CGT AGA CAC TGG GCT TTT GGG GAC ATT CCC TGC CGA GTG     270

  91  G   L   F   T   L   A   M   N   R   A   G   S   I   V   F     105
 271 GGG CTC TTC ACG TTG GCC ATG AAC AGG GCC GGG AGC ATC GTG TTC     315

 106  L   T   V   V   A   A   D   R   Y   F   K   V   V   H   P     120
 316 CTT ACG GTG GTG GCT GCG GAC AGG TAT TTC AAA GTG GTC CAC CCC     360

 121  H   H   A   V   N   T   I   S   T   R   V   A   A   G   I     135
 361 CAC CAC GCG GTG AAC ACT ATC TCC ACC CGG GTG GCG GCT GGC ATC     405

 136  V   C   T   L   W   A   L   V   I   L   G   T   V   Y   L     150
 406 GTC TGC ACC CTG TGG GCC CTG GTC ATC CTG GGA ACA GTG TAT CTT     450

 151  L   L   E   N   H   L   C   V   Q   E   T   A   V   S   C     165
 451 TTG CTG GAG AAC CAT CTC TGC GTG CAA GAG ACG GCC GTC TCC TGT     495

 166  E   S   F   I   M   E   S   A   N   G   W   H   D   I   M     180
 496 GAG AGC TTC ATC ATG GAG TCG GCC AAT GGC TGG CAT GAC ATC ATG     540

 181  F   Q   L   E   F   F   M   P   L   G   I   I   L   F   C     195
 541 TTC CAG CTG GAG TTC TTT ATG CCC CTC GGC ATC ATC TTA TTT TGC     585

 196  S   F   K   I   V   W   S   L   R   R   R   Q   Q   L   A     210
 586 TCC TTC AAG ATT GTT TGG AGC CTG AGG CGG AGG CAG CAG CTG GCC     630

 211  R   Q   A   R   M   K   K   A   T   R   F   I   M   V   V     225
 631 AGA CAG GCT CGG ATG AAG AAG GCG ACC CGG TTC ATC ATG GTG GTG     675

 226  A   I   V   F   I   T   C   Y   L   P   S   V   S   A   R     240
 676 GCA ATT GTG TTC ATC ACA TGC TAC CTG CCC AGC GTG TCT GCT AGA     720

 241  L   Y   F   L   W   T   V   P   S   S   A   C   D   P   S     255
 721 CTC TAT TTC CTC TGG ACG GTG CCC TCG AGT GCC TGC GAT CCC TCT     765
```

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 271  S   M   L   D   P   L   V   Y   Y   F   S   S   P   S   F     285
 811 AGC ATG CTG GAT CCC CTG GTG TAT TAT TTT TCA AGC CCC TCC TTT     855

 286  P   K   F   Y   N   K   L   K   I   C   S   L   K   P   K     300
 856 CCC AAA TTC TAC AAC AAG CTC AAA ATC TGC AGT CTG AAA CCC AAG     900

 301  Q   P   G   H   S   K   T   Q   R   P   E   E   M   P   I     315
 901 CAG CCA GGA CAC TCA AAA ACA CAA AGG CCG GAA GAG ATG CCA ATT     945

 316  S   N   L   G   R   R   S   C   I   S   V   A   N   S   F     330
 946 TCG AAC CTC GGT CGC AGG AGT TGC ATC AGT GTG GCA AAT AGT TTC     990

 331  Q   S   Q   S   D   G   Q   W   D   P   H   I   V   E   W     345
 991 CAA AGC CAG TCT GAT GGG CAA TGG GAT CCC CAC ATT GTT GAG TGG    1035

 346  H   *                                                       347
1036 CAC TGA                                                     1041
```

Amino acid sequence of human GPCRx14 (346 amino acids) (SEQ ID NO:12). The seven predicted transmembrane domaines are underlined.

MYNGSCCRIEGDTISQVMPPLLIVAFVLGALGNGVALCGFCFHMKTWKPSTVYLFNLAVADFLLMICLPFRTDYYLRRRH
WAFGDIPCRVGLFTLAMNRAGSIVFLTVVAADRYFKVVHPHHAVNTISTRVAAGIVCTLWALVILGTVYLLLENHLCVQE
TAVSCESFIMESANGWHDIMFQLEFFMPLGIILFCSFKIVWSLRRRQQLARQARMKKATRFIMVVAIVFITCYLPSVSAR
LYFLWTVPSSACDPSVHGALHITLSFTYMNSMLDPLVYYFSSPSFPKFYNKLKICSLKPKQPGHSKTQRPEEMPISNLGR
RSCISVANSFQSQSDGQWDPHIVEWH

At the amino acid sequence level, the human GPCRx14 is 50% identical to the human HM74 receptor.

Nucleotide and deduced amino acid sequence of human GPCRx16 (SEQ ID NO: 13 and 14 respectively). This nucleotide sequence is located on the chromosome 4.

```
   1  M   G   P   G   E   A   L   L   A   G   L   L   V   M   V      15
   1 ATG GGC CCC GGC GAG GCG CTG CTG GCG GGT CTC CTG GTG ATG GTA      45

  16  L   A   V   A   L   L   S   N   A   L   V   L   L   C   C      30
  46 CTG GCC GTG GCG CTG CTA TCC AAC GCA CTG GTG CTG CTT TGT TGC      90

  31  A   Y   S   A   E   L   R   T   R   A   S   G   V   L   L      45
  91 GCC TAC AGC GCT GAG CTC CGC ACT CGA GCC TCA GGC GTC CTC CTG     135

  46  V   N   L   S   L   G   H   L   L   L   A   A   L   D   M      60
 136 GTG AAT CTG TCT CTG GGC CAC CTG CTG CTG GCG GCG CTG GAC ATG     180

  61  P   F   T   L   L   G   V   M   R   G   R   T   P   S   A      75
 181 CCC TTC ACG CTG CTC GGT GTG ATG CGC GGG CGG ACA CCG TCG GCG     225

  76  P   G   A   C   Q   V   I   G   F   L   D   T   F   L   A      90
 226 CCC GGC GCA TGC CAA GTC ATT GGC TTC CTG GAC ACC TTC CTG GCG     270

  91  S   N   A   A   L   S   V   A   A   L   S   A   D   Q   W     105
 271 TCC AAC GCG GCG CTG AGC GTG GCG GCG CTG AGC GCA GAC CAG TGG     315

 106  L   A   V   G   F   P   L   R   Y   A   G   R   L   R   P     120
 316 CTG GCA GTG GGC TTC CCA CTG CGC TAC GCC GGA CGC CTG CGA CCG     360

 121  R   Y   A   G   L   L   L   G   C   A   W   G   Q   S   L     135
 361 CGC TAT GCC GGC CTG CTG CTG GGC TGT GCC TGG GGA CAG TCG CTG     405

 136  A   F   S   G   A   A   L   G   C   S   W   L   G   Y   S     150
 406 GCC TTC TCA GGC GCT GCA CTT GGC TGC TCG TGG CTT GGC TAC AGC     450

 151  S   A   F   A   S   C   S   L   R   L   P   P   E   P   E     165
 451 AGC GCC TTC GCG TCC TGT TCG CTG CGC CTG CCG CCC GAG CCT GAG     495

 166  R   P   R   F   A   A   F   T   A   T   L   H   A   V   G     180
 496 CGT CCG CGC TTC GCA GCC TTC ACC GCC ACG CTC CAT GCC GTG GGC     540

 181  F   V   L   P   L   A   V   L   C   L   T   S   L   Q   V     195
 541 TTC GTG CTG CCG CTG GCG GTG CTC TGC CTC ACC TCG CTC CAG GTG     585

 196  H   R   V   A   R   R   H   C   Q   R   M   D   T   V   T     210
 586 CAC CGG GTG GCA CGC AGA CAC TGC CAG CGC ATG GAC ACC GTC ACC     630

 211  M   K   A   L   A   L   L   A   D   L   H   P   R   Y   W     225
 631 ATG AAG GCG CTC GCG CTG CTC GCC GAC CTG CAC CCC AGG TAT TGG     675

 226  P   S   A   C   R   Q   A   Q   A   R   D   L   G   A   P     240
 676 CCC AGT GCA TGC CGA CAG GCC CAG GCC AGG GAC TTG GGC GCT CCC     720

 241  W   A   V   G   L   R   S   L   W   A   S   P   P   L   L     255
 721 TGG GCA GTT GGC TTG AGG AGC CTG TGG GCA TCA CCA CCG TTA CTC     765

 256  C   P   E   F   T   S   H   S   T   A   P   A   R   C   S     270
```

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271  Q   G   F   P   V   G   S   L   V   Q   T   L   R   G   P      285
811 CAG GGG TTT CCT GTT GGT TCA TTG GTG CAG ACA CTG CGG GGG CCT     855

286  L   P   P   G   I   C   A   H   S   A   Q   G   A   L   R      300
856 CTG CCT CCT GGG ATA TGT GCT CAC AGT GCA CAG GGA GCT TTG CGC     900

301  R   A   V   G   C   A   S   P   G   G   V   P   R   A   L      315
901 AGA GCT GTG GGG TGT GCT TCT CCG GGA GGG GTT CCG CGG GCT CTG     945

316  L   W   A   A   R   H   T   P   P   V   H   G   C   G   S      330
946 CTG TGG GCG GCC AGA CAC ACC CCT CCT GTG CAT GGC TGT GGG TCT     990

331  E   A   S   A   C   F   C   P   L   L   T   Q   C   P   C      345
991 GAG GCA TCT GCT TGT TTC TGC CCA CTG CTG ACC CAG TGC CCT TGC    1035

346  M   D   L   G   F   K   S   *                                  352
1036 ATG GAC TTG GGC TTC AAG TCT TGA                               1059
```

Amino acid sequence of human GPCRx16 (352 amino acids) (SEQ ID NO: 14). The six predicted transmembrane domaines are underlined.

MGPGEALLAGLLVMVLAVALLSNALVLLCCAYSAELRTRASGVLLVNLSLGHLLLAALDMPFTLLGVMRGRTPSAPGACQ
VIGFLDTFLASNAALSVAALSADQWLAVGFPLRYAGRLRPRYAGLLLGCAWGQSLAFSGAALGCSWLGYSSAFASCSLRL
PPEPERPRFAAFTATLHAVGFVLPLAVLCLTSLQVHRVARRHCQRMDTVTMKALALLADLHPRYWPSACRQAQARDLGAP
WAVGLRSLWASPPLLCPEFTSHSTAPARCSQGFPVGSLVQTLRGPLPPGICAHSAQGALRRAVGCASPGGVPRALLWAAR
HTPPVHGCGSEASACFCPLLTQCPCMDLGFKS

At the amino acid sequence level, the human GPCRx16 is 50% identical to the rat GPR 26 receptor.

Nucleotide and deduced amino acid sequence of human GPCRx17 (SEQ ID NO: 15 and 16 respectively). This nucleotide sequence is located on the chromosome 2.

```
  1  M   T   P   N   S   T   G   E   V   P   S   P   I   P   K      15
  1 ATG ACG CCC AAC AGC ACT GGC GAG GTG CCC AGC CCC ATT CCC AAG      45

 16  G   A   L   G   L   S   L   A   L   A   S   L   I   I   T      30
 46 GGG GCT TTG GGG CTC TCC CTG GCC CTG GCA AGC CTC ATC ATC ACC      90

 31  A   N   L   L   L   A   L   G   I   A   G   T   A   A   C      45
 91 GCG AAC CTG CTC CTA GCC CTG GGC ATC GCT GGG ACC GCC GCC TGC     135

 46  A   A   T   C   W   L   L   L   P   E   P   T   A   G   W      60
136 GCA GCC ACC TGC TGG CTG CTT CTT CCT GAG CCT ACT GCT GGC TGG     180

 61  A   A   H   G   S   G   I   A   T   L   P   G   L   W   N      75
181 GCT GCT CAC GGG TCT GGC ATT GCC ACA TTG CCA GGG CTG TGG AAC     225

 76  Q   S   R   R   G   Y   W   S   C   L   L   V   Y   L   A      90
226 CAG AGT CGC CGG GGT TAC TGG TCC TGC CTC CTC GTC TAC TTG GCT     270

 91  P   N   F   S   F   L   S   L   L   A   N   L   L   L   V     105
271 CCC AAC TTC TCC TTC CTC TCC CTG CTT GCC AAC CTC TTG CTG GTG     315

106  H   G   E   R   Y   M   A   V   L   R   P   L   Q   P   P     120
316 CAC GGG GAG CGC TAC ATG GCA GTC CTG AGG CCA CTC CAG CCC CCT     360

121  G   S   I   R   L   A   L   L   L   T   W   A   G   P   L     135
361 GGG AGC ATT CGG CTG GCC CTG CTC CTC ACC TGG GCT GGT CCC CTG     405

136  L   F   A   S   L   P   A   L   G   W   N   H   W   T   P     150
406 CTC TTT GCC AGT CTG CCC GCT CTG GGG TGG AAC CAC TGG ACC CCT     450

151  G   A   N   C   S   S   Q   A   I   F   P   A   P   Y   L     165
451 GGT GCC AAC TGC AGC TCC CAG GCT ATC TTC CCA GCC CCC TAC CTG     495

166  Y   L   E   V   Y   G   L   L   L   P   A   V   G   A   A     180
496 TAC CTC GAA GTC TAT GGG CTC CTG CTG CCC GCC GTG GGT GCT GCT     540

181  A   F   L   S   V   R   V   L   A   T   A   H   R   Q   L     195
541 GCC TTC CTC TCT GTC CGC GTG CTG GCC ACT GCC CAC CGC CAG CTG     585

196  Q   D   I   C   R   L   E   R   A   V   C   R   D   E   P     210
586 CAG GAC ATC TGC CGG CTG GAG CGG GCA GTG TGC CGC GAT GAG CCC     630

211  S   A   L   A   R   A   L   T   W   R   Q   A   R   A   Q     225
631 TCC GCC CTG GCC CGG GCC CTT ACC TGG AGG CAG GCA AGG GCA CAG     675

226  A   G   A   M   L   L   F   G   L   C   W   G   P   Y   V     240
676 GCT GGA GCC ATG CTG CTC TTC GGG CTG TGC TGG GGG CCC TAC GTG     720

241  A   T   L   L   L   S   V   L   A   Y   E   Q   R   P   P     255
721 GCC ACA CTG CTC CTC TCA GTC CTG GCC TAT GAG CAG CGC CCG CCA     765
```

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271  S   A   A   A   V   P   V   A   M   G   L   G   D   Q   R      285
811 AGT GCA GCG GCA GTG CCC GTA GCC ATG GGG CTG GGC GAT CAG CGC     855

286  Y   T   A   P   W   R   Q   P   P   K   G   A   C   R   G      300
856 TAC ACA GCC CCC TGG AGG CAG CCG CCC AAA GGT GCC TGC AGG GGC     900

301  C   G   E   E   P   P   G   T   V   P   A   P   A   L   P      315
901 TGT GGG GAA GAG CCT CCC GGG ACA GTC CCG GCC CCA GCA TTG CCT     945

316  T   T   Q   A   A   K   A   V   S   T   W   T   *              327
946 ACC ACC CAA GCA GCC AAA GCA GTG TCG ACC TGG ACT TGA             984
```

Amino acid sequence of human GPCRx17 (327 amino acids) (SEQ ID NO:16). The seven predicted transmembrane domaines are underlined.

MTPNSTGEVPSPIPKGALGLSLALASLIITANLLLALGIAGTAACAATCWLLLPEPTAGWAAHGSGIATLPGLWNQSRRG
YWSCLLVYLAPNFSFLSLLANLLLVHGERYMAVLRPLQPPGSIRLALLLTWAGPLLFASLPALGWNHWTPGANCSSQAIF
PAPYLYLEVYGLLLPAVGAAAFLSVRVLATAHRQLQDICRLERAVCRDEPSALARALTWRQARAQAGAMLLFGLCWGPYV
ATLLLSVLAYEQRPPLGPGTLLSLLSLGSASAAAVPVAMGLCDQRYTAPWRQPPKGACRGCGEEPPGTVPAPALPTTQAA
KAVSTWT

At the amino acid sequence level, the human GPCRx17 is 28% identical to the human EDG6 receptor

Nucleotide and deduced amino acid sequence of human GPCRx18 (SEQ ID NO: 17 and 18 respectively). This nucleotide sequence is located on the chromosome 2.

```
   1 M  G  D  E  L  A  P  C  P  V  G  T  T  A  W      15
   1 ATG GGG GAT GAG CTG GCA CCT TGC CCT GTG GGC ACT ACA GCT TGG      45

  16 P  A  L  I  Q  L  I  S  K  T  P  C  M  P  Q      30
  46 CCG GCC CTG ATC CAG CTC ATC AGC AAG ACA CCC TGC ATG CCC CAA      90

  31 A  A  S  N  T  S  L  G  L  G  D  L  R  V  P      45
  91 GCA GCC AGC AAC ACT TCC TTG GGC CTG GGG GAC CTC AGG GTG CCC     135

  46 S  S  M  L  Y  W  L  F  L  P  S  S  L  L  A      60
 136 AGC TCC ATG CTG TAC TGG CTT TTC CTT CCC TCA AGC CTG CTG GCT     180

  61 A  A  T  L  A  V  S  P  L  L  L  V  T  I  L      75
 181 GCA GCC ACA CTG GCT GTC AGC CCC CTG CTG CTG GTG ACC ATC CTG     225

  76 R  N  Q  R  L  R  Q  E  P  H  Y  L  L  P  A      90
 226 CGG AAC CAA CGG CTG CGA CAG GAG CCC CAC TAC CTG CTC CCG GCT     270

  91 N  I  L  L  S  D  L  A  Y  I  L  L  H  M  L     105
 271 AAC ATC CTG CTC TCA GAC CTG GCC TAC ATT CTC CTC CAC ATG CTC     315

 106 I  S  S  S  S  L  G  G  W  E  L  G  R  M  A     120
 316 ATC TCC TCC AGC AGC CTG GGT GGC TGG GAG CTG GGC CGC ATG GCC     360

 121 C  G  I  L  T  D  A  V  F  A  A  C  T  S  T     135
 361 TGT GGC ATT CTC ACT GAT GCT GTC TTC GCC GCC TGC ACC AGC ACC     405

 136 I  L  S  F  T  A  I  V  L  H  T  Y  L  A  V     150
 406 ATC CTG TCC TTC ACC GCC ATT GTG CTG CAC ACC TAC CTG GCA GTC     450

 151 I  H  P  L  R  Y  L  S  F  M  S  H  G  A  A     165
 451 ATC CAT CCA CTG CGC TAC CTC TCC TTC ATG TCC CAT GGG GCT GCC     495

 166 W  K  A  V  A  L  I  W  L  V  A  C  C  F  P     180
 496 TGG AAG GCA GTG GCC CTC ATC TGG CTG GTG GCC TGC TGC TTC CCC     540

 181 T  F  L  I  W  L  S  K  W  Q  D  A  Q  L  E     195
 541 ACA TTC CTT ATT TGG CTC AGC AAG TGG CAG GAT GCC CAG CTG GAG     585

 196 E  Q  G  A  S  Y  I  L  P  P  S  M  G  T  Q     210
 586 GAG CAA GGA GCT TCA TAC ATC CTA CCA CCA AGC ATG GGC ACC CAG     630

 211 P  G  C  G  L  L  V  I  V  T  Y  T  S  I  L     225
 631 CCG GGA TGT GGC CTC CTG GTC ATT GTT ACC TAC ACC TCC ATT CTG     675

 226 C  V  L  F  L  C  T  A  L  I  A  N  C  F  W     240
 676 TGC GTT CTG TTC CTC TGC ACA GCT CTC ATT GCC AAC TGT TTC TGG     720

 241 R  I  Y  A  E  A  K  T  S  G  I  W  G  Q  G     255
 721 AGG ATC TAT GCA GAG GCC AAG ACT TCA GGC ATC TGG GGG CAG GGC     765
```

```
271  T   L   Y   V   S   T   G   V   V   F   S   L   D   M   V      285
811 ACA TTG TAC GTG AGC ACA GGG GTG GTG TTC TCC CTG GAC ATG GTG     855

286  L   T   R   Y   H   H   I   D   S   G   T   H   T   W   L      300
856 CTG ACC AGG TAC CAC CAC ATT GAC TCT GGG ACT CAC ACA TGG CTC     900

301  L   A   A   N   S   E   V   L   M   M   L   P   R   A   M      315
901 CTG GCA GCT AAC AGT GAG GTA CTC ATG ATG CTT CCC CGT GCC ATG     945

316  L   T   Y   L   Y   L   L   R   Y   R   Q   L   L   G   M      330
946 CTC ACA TAC CTG TAC CTG CTC CGC TAC CGG CAG CTG TTG GGC ATG     990

331  V   R   G   H   L   P   S   R   R   H   Q   A   I   F   T      345
991 GTC CGG GGC CAC CTC CCA TCC AGG AGG CAC CAG GCC ATC TTT ACC    1035

346  I   S   *                                                      347
1036 ATT TCC TAG                                                   1044
```

Amino acid sequence of human GPCRx18 (347 amino acids) (SEQ ID NO:18). The seven predicted transmembrane domaines are underlined.

MGDELAPCPVGTTAWPALIQLISKTPCMPQAASNTSLGLGDLRVPSSMLYWLFLPSSLLAAATLAVSPLLLVTILRNQRL
RQEPHYLLPANILLSDLAYILLHMLISSSSLGGWELGRMACGILTDAVFAACTSTILSFTAIVLHTYLAVIHPLRYLSFM
SHGAAWKAVALIWLVACCFPTFLIWLSKWQDAQLEEQGASYILPPSMGTQPGCGLLVIVTYTSILCVLFLCTALIANCFW
RIYAEAKTSGIWGQGYSRARGTLLIHSVLITLYVSTGVVFSLDMVLTRYHHIDSGTHTWLLAANSEVLMMLPRAMLTYLY
LLRYRQLLGMVRGHLPSRRHQAIFTIS

At the amino acid sequence level, the human GPCRx18 is 25% identical to the rabbit 5HT1D-β receptor.

Nucleotide and deduced amino acid sequence (partial sequence) of human GPCRx19 (SEQ ID NO: 19 and 20 respectively). This nucleotide sequence is located on the chromosome 16.

```
  1 G   P   H   R   S   Q   R   S   H   L   C   F   R   A   K      15
  1 GGC CCC CAT AGG AGC CAA CGA AGT CAT CTT TGC TTC AGA GCT AAA      45

 16 P   V   F   L   L   S   T   A   N   I   L   T   V   I   I      30
 46 CCA GTT TTT CTT CTC TCC ACA GCA AAT ATC TTG ACA GTG ATC ATC      90

 31 L   S   Q   L   V   A   R   R   Q   K   S   S   Y   N   Y      45
 91 CTC TCC CAG CTG GTG GCA AGA AGA CAG AAG TCC TCC TAC AAC TAT     135

 46 L   L   A   L   A   A   A   D   I   L   V   L   F   F   I      60
136 CTC TTG GCA CTC GCT GCT GCC GAC ATC TTG GTC CTC TTT TTC ATA     180

 61 V   F   V   D   F   L   L   E   D   F   I   L   N   M   Q      75
181 GTG TTT GTG GAC TTC CTG TTG GAA GAT TTC ATC TTG AAC ATG CAG     225

 76 M   P   Q   V   P   D   K   I   I   E   V   L   E   F   S      90
226 ATG CCT CAG GTC CCC GAC AAG ATC ATA GAA GTG CTG GAA TTC TCA     270

 91 S   I   H   T   S   I   W   I   T   V   P   L   T   I   D     105
271 TCC ATC CAC ACC TCC ATA TGG ATT ACT GTA CCG TTA ACC ATT GAC     315

106 R   Y   I   A   V   C   H   P   L   K   Y   H   T   V   S     120
316 AGG TAT ATC GCT GTC TGC CAC CCG CTC AAG TAC CAC ACG GTC TCA     360

121 Y   P   A   R   T   R   K   V   I   V   S   V   Y   I   T     135
361 TAC CCA GCC CGC ACC CGG AAA GTC ATT GTA AGT GTT TAC ATC ACC     405

136 C   F   L   T   S   I   P   Y   Y   W   W   P   N   I   W     150
406 TGC TTC CTG ACC AGC ATC CCC TAT TAC TGG TGG CCC AAC ATC TGG     450

151 T   E   D   Y   I   S   T   S   V   H   H   V   L   I   W     165
451 ACT GAA GAC TAC ATC AGC ACC TCT GTG CAT CAC GTC CTC ATC TGG     495

166 I   H   C   F   T   V   Y   L   V   P   C   S   I   F   F     180
496 ATC CAC TGC TTC ACC GTC TAC CTG GTG CCC TGC TCC ATC TTC TTC     540

181 I   L   N   S   I   I   V   Y   K   L   R   R   K   S   N     195
541 ATC TTG AAC TCA ATC ATT GTG TAC AAG CTC AGG AGG AAG AGC AAT     585

196 F   R   L   R   G   Y   S   T   G   K   T   T   A   I   L     210
586 TTT CGT CTC CGT GGC TAC TCC ACG GGG AAG ACC ACC GCC ATC TTG     630

211 F   T   I   T   S   I   F   A   T   L   W   A   P   R   I     225
631 TTC ACC ATT ACC TCC ATC TTT GCC ACA CTT TGG GCC CCC CGC ATC     675

226 I   M   I   L   Y   H   L   Y   G   A   P   I   Q   N   R     240
676 ATC ATG ATT CTT TAC CAC CTC TAT GGG GCG CCC ATC CAG AAC CGC     720

241 W   L   V   H   I   M   S   D   I   A   N   M   L   A   L     255
721 TGG CTG GTA CAC ATC ATG TCC GAC ATT GCC AAC ATG CTA GCC CTT     765
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271  R   F   R   T   M   A   A   A   T   L   K   A   F   F   K     285
811  CGG TTC CGC ACC ATG GCA GCC GCC ACG CTC AAG GCT TTC TTC AAG     855

286  C   Q   K   Q   P   V   Q   F   Y   T   N   H   N   F   S     300
856  TGC CAG AAG CAA CCT GTA CAG TTC TAC ACC AAT CAT AAC TTT TCC     900

301  I   T   S   S   P   W   I   S   P   A   N   S   H   C   I     315
901  ATA ACA AGT AGC CCC TGG ATC TCG CCG GCA AAC TCA CAC TGC ATC     945

316  K   M   L   V   Y   Q   Y   D   K   N   G   K   P   I   K     330
946  AAG ATG CTG GTG TAC CAG TAT GAC AAA AAT GGA AAA CCT ATA AAA     990

331  V   S   P   *                                                 333
991  GTA TCC CCG TGA                                               1002
```

Partial amino acid sequence of human GPCRx19 (333 amino acids) (SEQ ID NO:20). The seven predicted transmembrane domaines are underlined.

GPHRSQRSHLCFRAKPVFLLSTANILTVIILSQLVARRQKSSYNYLLALAAADILVLFFIVFVDFLLEDFILNMQMPQVP
DKLIEVLEFSSIHTSIWITVPLTIDRYIAVCHPLKYHTVSYPARTRKVIVSVYITCFLTSIPYYWWPNIWTEDYISTSVH
HVLIWIHCFTVYLVPCSIFFILNSIIVYKLRRKSNFRLRGYSTGKTTAILFTITSIFATLWAPRIIMILYHLYGAPIQNR
WLVHIMSDIANMLALLNTAINFFLYCFISKRFRTMAAATLKAFFKCQKQPVQFYTNHNFSITSSPWISPANSHCIKMLVY
QYDKNGKPIKVSP

At the amino acid sequence level, the human GPCRx19 is 25% identical to the *C. Elegans* F21C10.9 G-protein coupled receptor.

Nucleotide and deduced amino acid sequence of human GPCRx20 (SEQ ID NO: 21 and 22 respectively). This nucleotide sequence is located on the chromosome 5.

```
   1 M   L   A   A   A   F   A   D   S   N   S   S   S   M   N      15
   1 ATG CTG GCA GCT GCC TTT GCA GAC TCT AAC TCC AGC AGC ATG AAT      45

  16 V   S   F   A   H   L   H   F   A   G   G   Y   L   P   S      30
  46 GTG TCC TTT GCT CAC CTC CAC TTT GCC GGA GGG TAC CTG CCC TCT      90

  31 D   S   Q   D   W   R   T   I   I   P   A   L   L   V   A      45
  91 GAT TCC CAG GAC TGG AGA ACC ATC ATC CCG GCT CTC TTG GTG GCT     135

  46 V   C   L   V   G   F   V   G   N   L   C   V   I   G   I      60
 136 GTC TGC CTG GTG GGC TTC GTG GGA AAC CTG TGT GTG ATT GGC ATC     180

  61 L   L   H   N   A   W   K   G   K   P   S   M   I   H   S      75
 181 CTC CTT CAC AAT GCT TGG AAA GGA AAG CCA TCC ATG ATC CAC TCC     225

  76 L   I   L   N   L   S   L   A   D   L   S   L   L   L   F      90
 226 CTG ATT CTG AAT CTC AGC CTG GCT GAT CTC TCC CTC CTG CTG TTT     270

  91 S   A   P   I   R   A   T   A   Y   S   K   S   V   W   D     105
 271 TCT GCA CCT ATC CGA GCT ACG GCG TAC TCC AAA AGT GTT TGG GAT     315

 106 L   G   W   F   V   C   K   S   S   D   W   F   I   H   T     120
 316 CTA GGC TGG TTT GTC TGC AAG TCC TCT GAC TGG TTT ATC CAC ACA     360

 121 C   M   A   A   K   S   L   T   I   V   V   V   A   K   V     135
 361 TGC ATG GCA GCC AAG AGC CTG ACA ATC GTT GTG GTG GCC AAA GTA     405

 136 C   F   M   Y   A   S   D   P   A   K   Q   V   S   I   H     150
 406 TGC TTC ATG TAT GCA AGT GAC CCA GCC AAG CAA GTG AGT ATC CAC     450

 151 N   Y   T   I   W   S   V   L   V   A   I   W   T   V   A     165
 451 AAC TAC ACC ATC TGG TCA GTG CTG GTG GCC ATC TGG ACT GTG GCT     495

 166 S   L   L   P   L   P   E   W   F   F   S   T   I   R   H     180
 496 AGC CTG TTA CCC CTG CCG GAA TGG TTC TTT AGC ACC ATC AGG CAT     540

 181 H   E   G   V   E   M   C   L   V   D   V   P   A   V   A     195
 541 CAT GAA GGT GTG GAA ATG TGC CTC GTG GAT GTA CCA GCT GTG GCT     585

 196 E   E   F   M   S   M   F   G   K   L   Y   P   L   L   A     210
 586 GAA GAG TTT ATG TCG ATG TTT GGT AAG CTC TAC CCA CTC CTG GCA     630

 211 F   G   L   P   L   F   F   A   S   F   Y   F   W   R   A     225
 631 TTT GGC CTT CCA TTA TTT TTT GCC AGC TTT TAT TTC TGG AGA GCT     675

 226 Y   D   Q   C   K   K   R   G   T   K   T   Q   N   L   R     240
 676 TAT GAC CAA TGT AAA AAA CGA GGA ACT AAG ACT CAA AAT CTT AGA     720

 241 N   Q   I   R   S   K   Q   V   T   V   M   L   L   S   I     255
 721 AAC CAG ATA CGC TCA AAG CAA GTC ACA GTG ATG CTG CTG AGC ATT     765

 256 A   I   I   S   A   L   L   W   L   P   F   W   V   A   W     270
```

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 271  L   W   V   W   H   L   K   A   A   G   P   A   P   P   Q      285
 811 CTG TGG GTA TGG CAT CTG AAG GCT GCA GGC CCG GCC CCA CCA CAA     855

 286  G   F   I   A   L   S   Q   V   L   M   F   S   I   S   S      300
 856 GGT TTC ATA GCC CTG TCT CAA GTC TTG ATG TTT TCC ATC TCT TCA     900

 301  A   N   P   L   I   F   L   V   M   S   E   E   F   R   E      315
 901 GCA AAT CCT CTC ATT TTT CTT GTG ATG TCG GAA GAG TTC AGG GAA     945

 316  G   L   K   G   V   W   K   W   M   I   T   K   K   P   P      330
 946 GGC TTG AAA GGT GTA TGG AAA TGG ATG ATA ACC AAA AAA CCT CCA     990

 331  T   V   S   E   S   Q   E   T   P   A   G   N   S   E   G      345
 991 ACT GTC TCA GAG TCT CAG GAA ACA CCA GCT GGC AAC TCA GAG GGT    1035

 346  L   P   D   K   V   P   S   P   E   S   P   A   S   I   P      360
1036 CTT CCT GAC AAG GTT CCA TCT CCA GAA TCC CCA GCA TCC ATA CCA    1080

 361  E   K   E   K   P   S   S   P   S   S   G   K   G   K   T      375
1081 GAA AAA GAG AAA CCC AGC TCT CCC TCC TCT GGC AAA GGG AAA ACT    1125

 376  E   K   A   E   I   P   I   L   P   D   V   E   Q   F   W      390
1126 GAG AAG GCA GAG ATT CCC ATC CTT CCT GAC GTA GAG CAG TTT TGG    1170

 391  H   E   R   D   T   V   P   S   V   Q   D   N   D   P   I      405
1171 CAT GAG AGG GAC ACA GTC CCT TCT GTA CAG GAC AAT GAC CCT ATC    1215

 406  P   W   E   H   E   D   Q   E   T   G   E   G   V   K   *      419
1216 CCC TGG GAA CAT GAA GAT CAA GAG ACA GGG GAA GGT GTT AAA TAG    1260
```

Amino acid sequence of human GPCRx20 (419 amino acids) (SEQ ID NO:22). The seven predicted transmembrane domaines are underlined.

MLAAAFADSNSSSMNVSFAHLHFAGGYLPSDSQDWRTIIPALLVAVCLVGFVGNLCVIGILLHNAWKGKPSMIHSlILNL
SLADLSLLLFSAPIRATAYSKSVWDLGWFVCKSSDWFIHTCMAAKSLTIVVVAKVCFMYASDPAKQVSIHNYTIWSVLVA
IWTVASLLPLPEWFFSTIRHHEGVEMCLVDVPAVAEEFMSMFGKLYPLLAFGLPLFFASFYFWRAYDQCKKRGTKTQNLR
NQIRSKQVTVMLLSIAIISALLWLPEWVAWLWVWHLKAAGPAPPQGFIALSQVLMFSISSANPLIFLVMSEEFREGLKGV
WKWMITKKPPTVSESQETPAGNSEGLPDKVPSPESPASIPEKEKPSSPSSGKGKTEKAEIPILPDVEQFWHERDTVPSVQ
DNDPIPWEHEDQETGEGVK

At the amino acid sequence level, the human GPCRx20 is 20% identical to the mouse galanin 2 receptor.

CLAIMS

1. A G-protein coupled receptor having an amino acid sequence which presents more than 75% sequence identity with the sequence SEQ ID NO. 1.

2. The G-protein coupled receptor according to claim 1, having an amino acid sequence which presents more than 80% sequence identity with the sequence SEQ ID NO. 1.

3. The G-protein coupled receptor according to claim 1, having an amino acid sequence which presents more than 85% sequence identity with the sequence SEQ ID NO. 1.

4. The G-protein coupled receptor according to claim 1, having an amino acid sequence which presents more than 90% sequence identity with the sequence SEQ ID NO. 1.

5. The G-protein coupled receptor according to claim 1, having an amino acid sequence which presents more than 95% sequence identity with the sequence SEQ ID NO. 1.

6. The G-protein coupled receptor having the amino acid sequence SEQ ID NO. 1 or a specific active portion thereof.

7. A polynucleotide encoding any of the amino acid sequences of the G-protein coupled receptor according to any of the preceding claims 1 to 6.

8. An agonist, reverse agonist, antagonist or inhibitor of the receptor or the polynucleotide according to any of the preceding claims 1 to 7.

9. A vector comprising the polynucleotide

according to the claim 9.

11. A non-human mammal comprising a partial or total deletion of the polynucleotide according to the claim 8 encoding the receptor according to any of the preceding claims 1 to 6, preferably an non-human mammal comprising an homologous recombination "knock-out" of said polynucleotide or a transgenic non-human mammal overexpressing above natural level said polynucleotide.

12. A method for the screening (detection and possibly recovering) of compounds or natural extract which are known or not known to be agonists, antagonists or inhibitors to the receptor according to any of the preceding claims 1 to 6, said method comprising :

- contacting a cell or cell extract from the cell transfected with a vector according to the claim 9,
- possibly isolating a membrane fraction from the cell extract or the complete cell with a compound binding to said receptor under conditions permitting binding of said compound or molecules present in said natural extract to said receptor, possibly by the activation of a functional response, and
- detecting the presence of any such compound or molecules by means of a bioassay (preferably a modification in the production of a second messenger or an increase in the receptor activity) in the presence of the other known compound working as an agonist, reverse agonist, antagonist or inhibitor to the receptor and thereby recovering and determining whether said unknown compound or molecule(s) is (are) able to work as an agonist, antagonist or inhibitor of the compound to its receptor.

13. An unknown compound or molecule(s), identified by the screening method according to the claim

an adequate pharmaceutical carrier and a sufficient amount

of the compound or molecules according to the claim 8 or 13.

15. Use of the pharmaceutical composition according to the claim 14, for the manufacture of a medicament in the prevention and/or the treatment of a disease selected from the group consisting of viral infections or diseases induced by various viruses or bacteria, the treatment of disturbances of cell migration, diseases or perturbations of the immune system, including cancer, development of tumours and tumour metastasis, inflammatory and neo-plastic processes, bacterial and fungal infections, for wound and bone healing and dysfunction of regulatory growth functions, pains, diabetes, obesity, anorexia, bulimia, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, restenosis, atherosclerosis, diseases characterised by excessive smooth muscle cell proliferation, aneurysms, wound healing, diseases characterised by loss of smooth muscle cells or reduced smooth muscle cell proliferation, stroke, ischemia, ulcers, allergies, benign prostatic hypertrophy, migraine, vomiting, psychotic and neurological disorders, including anxiety, schizophrenia, maniac depression, depression, delirium, dementia and severe mental retardation, degenerative diseases, neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, and dyskinasias, such as Huntington's disease or Gilles de la Tourett's syndrome and other related diseases.

16. Use of the pharmaceutical composition according to the claim 14, for the manufacture of a medicament in the prevention and/or the treatment of blood

17. Diagnostic kit comprising all the media and means for detecting the receptor and nucleotide sequence encoding it or an activity of said receptor and nucleotide sequence encoding it according to any of the preceding claims 1 to 8.

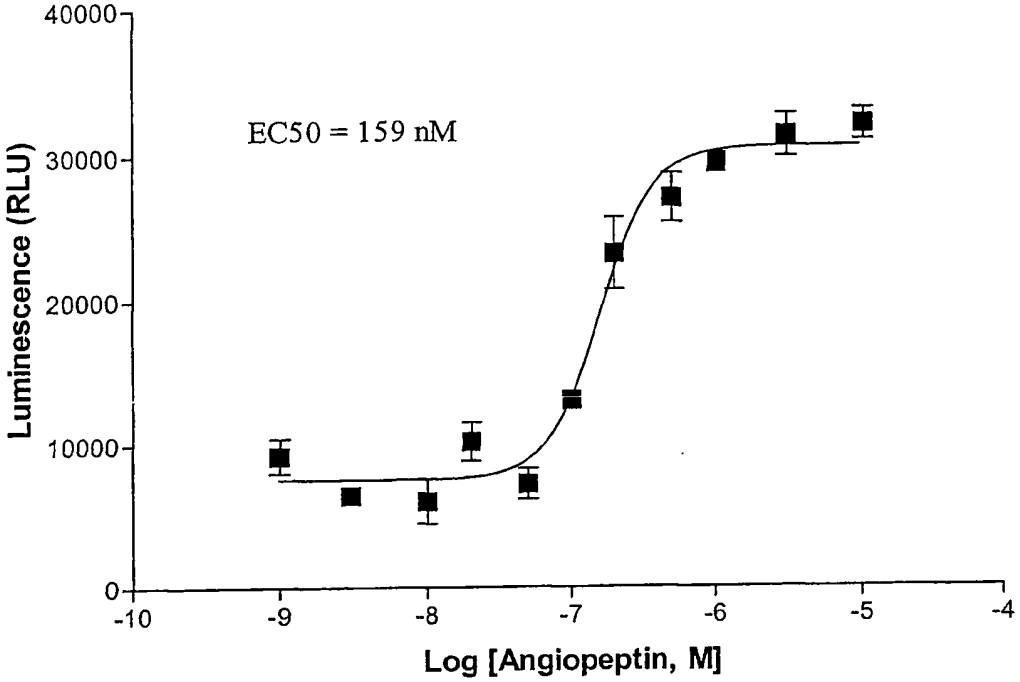

Figure 1 : Dose response curve with angiopeptin

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01870024.5	12 February 2001 (12.02.2001)	EP

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(74) Agents: **VAN MALDEREN, Eric** et al.; Office Van Malderen, Place Reine Fabiola 6/1, B-1083 Brussels (BE).

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/98330 A3

(54) Title: A RECOMBINANT CELL LINE EXPRESSING GPCRx11 AS A FUNCTIONAL RECEPTOR VALIDATED BY ANGIOPEPTIN AND USEFUL FOR SCREENING OF AGONISTS AND ANTAGONISTS

(57) Abstract: [illegible] reference ligands. GPCRx11 [illegible] specific signal with synthetic angiopeptin and a [illegible] assay. However, [illegible] this cell line for screening of natural or synthetic agonists and antagonists. In parallel, extended tissue distribution and polyclonal antibodies have been produced to facilitate GPCRx11 characterisation.

IN .RNATIONAL SEARCH REPORT

Inte. .ional Application No
PCT/BE 01/00104

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 16159 A (SMITHKLINE BEECHAM CORP) 8 March 2001 (2001-03-08) SEQ ID NO: 2; Claim 1 ---	1-7,9-12
P,X	WO 01 36471 A (ARENA PHARMACEUTICALS INC ;CHEN RUOPING (US); DANG HUONG T (US); L) 25 May 2001 (2001-05-25) SEQ ID NO: 30, Claim 57 ---	1-7,9-12
P,X	WO 01 19983 A (DELEERSNIJDER WILLY ;HEUVAERT NICOLE D (BE); NYS GUY (BE); SOLVAY) 22 March 2001 (2001-03-22) SEQ ID NO: 2, claims ---	1-7,9-12
P,X	WO 01 36473 A (PARODI LUIS A ;LIND PETER (SE); SEJLITZ TORSTEN (SE); SCHELLIN KAT) 25 May 2001 (2001-05-25) page 84, SEQ ID NO:57, Claim 37 ---	1-7,9-12

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

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1

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IN ..RNATIONAL SEARCH REPORT

Inte. ..ional Application No
PCT/BE 01/00104

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 01 48015 A (UPJOHN CO) 5 July 2001 (2001-07-05) Example 1, p. 85-85 (SEQ ID NO: 11) ---	1-7,9,10
E	WO 01 48189 A (MATSUMOTO SHUN ICHIRO ;MORIKAWA NORIYUKI (JP); SUGIYAMA TOMOYASU () 5 July 2001 (2001-07-05) SEQ ID NO: 44 ---	1-7,9,10
A	WO 99 32519 A (FORTIN YVES ;LEMBO PAOLA (CA); AHMAD SULTAN (CA); BANVILLE DENIS () 1 July 1999 (1999-07-01) -----	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 8, 13-17

Claims 8 and 13 refer to an agonist/antagonist of the polypeptide respectively to an unknown compound identified by the method of Claim 12 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 83 and 84 EPC). No search can be carried out for purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved. Consequently neither for Claims 14 to 16 which relate to the use of said compounds a search could be carried out.
The same, in principle applies for the "kit" of Claim 17 wherein none of the "media and means" which are essential for said kit are identified.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/BE 01/00104

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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